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# Impacts of temperature on life history traits of invasive Eastern Brook Trout ( Salvelinus fontinalis )

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# Impacts of Temperature on Life History Traits of Invasive Eastern Brook Trout (*Salvelinus fontinalis*)

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By Kaeli Alexis Davenport

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Acknowledgement of Data Collection

Data from Lime and Highline Creeks were provided by Madeline Lewis and Dr. Andrew Whiteley from the University of Montana. In order to show a more complete story of life-history of brook trout in the tributaries of the Pend Oreille we are including their data with permission. All analysis except for lipid content and HSP for Lime and Highline Creek was completed for all 8 streams and all methods used were standardized to ensure consistency in data collection across labs.

ACKNOWLEDGEMENTS

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## INTRODUCTION

Climate change is arguably the most important issue facing our world today. During the 20th century average global temperatures rose 0.85°C (IPCC 2014) and predictions of future conditions suggest larger and faster changes with some models projecting a possible 6°C increase within the next 100 years (IPCC 2007; Boer et al. 1992). As temperatures increase, impacts on natural ecosystems are expected to become more severe (Karl 2009). We may be able to slow or even correct some of these impacts, but others, including species extinctions and loss of coastal land due to rising sea levels, will be irreversible (Karl 2009).

Most climate change research focuses on the impacts on native species, but invasive species will respond to climate change as well (Hellman et al. 2008). For the purpose of this paper I will define invasive species as those that have a significant negative impact on native biota and ecosystems (Hellman et al. 2008). Climate change is expected to increase the likelihood of novel species invasions (Rahel and Olden 2008; Hellman et al. 2008). Negative impacts caused by invasive species may be exacerbated by climate change due to the fact that climate change is expected to alter the assemblage of potential invaders, influence the chance that non-native species can establish within new ecosystems, and alter their impact on the ecosystems they invade (Stachowicz et al. 2002; Rahel and Olden 2008). Many species that have the opportunity to invade, either by being transported accidentally or being introduced by humans, may not be able to successfully invade at the present due to unsuitable environments (Mooney and Cleland 2001). For example, *Mytilus galloprovincialis*, an invasive muscle from South Africa,

has been transported to Antarctica on shipping boats but has not successfully invaded due to the severe cold temperatures (Lee and Chown 2007). However, as water temperatures rise due to climate change, successful invasions could occur (Hellmann et al. 2008).

There are approximately 50,000 non-native species present within the United States and of these 50,000 species 4,300 of them are considered to be invasive (Pimentel et al. 2005). These 4,300 invasive species are the cause of about 42% of the species listed as threatened or endangered under the U.S. Endangered Species Act (Pimentel et al. 2005). As climate change impacts increase, these numbers are expected to grow, ultimately impacting biodiversity, displacing native species, and threatening the composition, structure and function of different ecosystems (Cortes et al. 2016). Among invasives, fish are especially numerous, costing the U.S. government more than $5.4 billion annually (Pimentel et al. 2005).

*Invasive Fish*

Non-native fish invasions have been a factor in 68% of fish extinctions in North America (Miller et al. 1989) and the rates of these invasions are increasing dramatically, impacting nearly every major watershed in the United States (EPA 2008). Fish can become invasive if they are introduced to new areas through intentional stocking or unintentional stocking (Rahel and Olden 2008). Success of an invasion is dependent upon the reproduction rate, growth, mortality, and successful competition of resources of the invading species with the native species (Sammarco et al. 2015). Invading fish species can cause different impacts on the ecosystem, in some cases completely displacing the

native species, in others becoming the dominant competitor for resources (Sammarco et al. 2015).

Climate change is expected to increase the likelihood of invasive fish species (Rahel and Olden 2008) by causing shifting environments that favor some species over others (Chown et al. 2007; Isaak et al. 2012). For example, climate change is expected to increase the suitable habitat for warmer water sport fish making future intentional introductions of specific species more likely (Rahel and Olden 2008; Hellmann et al. 2008). Climate change can also increase instances of invasive fish by increasing suitable habitats for specific aquaculture species. For example, the aquaculture of some fish species is expected to shift north as water temperatures rise and unfortunately, escapes of aquatic organisms from captive facilities can be common (Fuller et al. 1999; Orchard 1999; Padilla and Williams 2004). These escapees can then become invasive within the new habitat (Rahel and Olden 2008). The cold water boundary that prevents warmer water fish from establishing successful populations in colder water habitats is expected to weaken due to climate change. This could potentially cause stressful conditions for cold-water adapted fish species and allow warm-water adapted species to spread to these habitats and become established (Rahel and Olden 2008; Taniguchi et al. 1998; Sharma et al. 2007). For example, bull trout (*Salvelinus confluentus*), a threatened species in Northwestern United States, has an optimal temperature that is lower than that of other salmonids, giving them a competitive advantage over other non-native salmonids in colder water. However, climate change will cause thermally stressful environments for native bull trout, potentially allowing other salmonid species that have been introduced to invade and successfully compete (Nakano et al. 1996). Warming waters can also lead to

changes in competition success of different species. In Japan, native Dolly Varden (*Salvelinus malma*) are expected to be displaced by white-spotted charr (*Salvelinus leucomaenis*) as water temperatures increase due to the fact that white-spotted charr are superior competitors in warmer water temperatures (Rieman et al. 1997).

Models predicting future temperatures can be valuable for broad-scale predictions of future fish species invasions (Rahel 2002; Jackson and Mandrak 2002) but they mainly take into account environmental factors and fail to consider the sources of biological variation within invading species that could be driving the success of these species within novel habitats (Shrank et al. 2001; Mercado-Silva et al. 2006). In order to determine the impacts that climate change will have on invasive species we must first determine the biological characteristics that contribute to invasion success. Invasive fish can either respond plastically or adaptively to increase fitness within a new habitat. Responses that could increase invasion success include growth rate, time to reproduction, altered patterns of resource allocation, and improved stress response (Sammarco et al. 2015).

*Life-history*

For freshwater fishes, attributes that are directly related to species distributions include morphology, behavior, life-history, and habitat and trophic requirements. Of these attributes, life-history is considered to be a particularly good predictor for both fish invasions and extirpations (Olden et al. 2006). Life history traits include growth rate, age at maturation, and size at maturation and are directly involved in survival and reproduction (Cole 1954; Stearns and Koella 1986). These traits can be plastic, resulting in increased fitness for fish (Baker et al. 2015). According to Winemiller and Rose, there

are three possible strategies for life-history, and each is associated with a specific type of environment: 1) periodic strategists inhabit seasonal habitats and typically have larger bodies, mature later, and have more eggs per spawning event but have lower survivorship of juveniles (i.e. smaller eggs). 2) Opportunistic strategists inhabit unpredictable and highly disturbed habitats and are typically smaller, mature earlier, and have fewer eggs per spawning event as well as low survivorship of juveniles. 3) Equilibrium strategists inhabit predictable and stable habitats and are typically smaller to medium sized, mature at a moderate age, and have fewer eggs per spawning event but high survivorship of juveniles (larger eggs) (Winemiller and Rose 1992; Olden and Kennard 2010).

Based on the predictions of this model, fish life-history traits can be analyzed in three different ways: 1) size and age at maturation; 2) fecundity, defined as the number of eggs per female per spawning season, and 3) parental care or juvenile investment, defined as the size of eggs (Winemiller 1989; Mims and Olden 2012). Egg size directly influences offspring growth and survival and egg number influences female fitness. Each of these traits is directly linked to reproductive output (Baker et al. 2015) and both egg size and egg number can be direct results of female condition, i.e. length, weight, age and lipid content of females. Egg size and number vary among females and within females during different reproductive events (Bernardo 1996). Larger, older females tend to have either more or larger eggs, resulting in larger offspring, and a higher fitness of those offspring (Berkeley et al. 2004). For example, brown trout (*Salmo trutta*) that produce larger eggs spawn juveniles that experience growth and survival advantages over siblings of smaller eggs (Heath et al. 1999). In walleye (*Sander vitreus*), larger eggs contain more lipids and result in bigger larvae that are better able to withstand starvation

and escape predators compared to larvae from smaller eggs (Einum and Fleming 1999). In the mosquito fish (*Gambusia holbrooki*), development time, size of offspring, and gonopodium length in males (a primary male sexual character) all are impacted by maternal investment (Moodie et al. 1989). There is a trade-off between size and number of eggs because each female has a limited amount of resources that can be allocated to reproduction. Overall, females that have higher fecundity typically have smaller eggs resulting in a lower survivorship of eggs. In contrast, females that have lower fecundity will have larger eggs resulting in higher survivorship (Del Giudice et al. 2015).

Female condition (i.e. lipid content) can also impact female fitness. Reproduction requires energetic and physiological costs that can affect both current and future reproduction (Stearns 1992). Lipids are the major source of metabolic energy involved in reproduction in fish (Johnson 2009) and tend to mirror environmental resources. For example, decreases in food availability decrease available lipids which then can negatively affect different reproductive traits including time to maturity, fecundity and egg size (Zudairea et al. 2014). The 'cost of reproduction' can also influence future performance, affecting the probability of survival of reproducing individuals as well as future reproduction through reduced growth (Baker et al. 2015). If females are putting more resources into one reproduction event, this can impact the resources available for future reproduction.

Response to environmental stressors through rapid evolution, plasticity or both is an essential contribution of life-history traits. Plasticity in life-history traits within an invasive species could be highly beneficial. Plasticity is defined as the "ability of individual genotypes to produce different phenotypes when exposed to different

environmental conditions" (Pigliucci et al. 2006) and when associated with life-history it can provide the potential for organisms to respond rapidly and effectively to environmental change. Plasticity in life history is suggested as one reason for why some species can successfully invade and take advantage of new resources that novel habitats provide (Davidson et al. 2011; Pfennig et al. 2010).

Life-history traits in many species show extensive inducible plasticity where changes in the environment result in adjustments to life-history (Baker et al. 2015). For example, in a hybrid clonal fish (Poeciliidae: *Poeciliopsis)* increased density of fish has been shown to cause an increased time to maturity (Weeks and Quatro 1991) resulting in better competition for offspring. In three-spine stickleback (*Gasterosteus aculeatus*), high rations of food also increase time to maturity from average age 2 to 1 (Inness and Metcalfe, 2008). In some instances, plasticity in egg size can prepare offspring for the environmental conditions they will experience (Bernardo 1996). For instance, female three-spined stickleback exposed to the threat of predation produce larger eggs with higher cortisol content. These offspring then exhibit tighter shoaling behavior which is a known anti-predator defense (Vallin and Nissling 2000).

Life-history traits are considered valuable predictors of invasion success because they take into account traits that have consequences for fitness and are based on relationships between strategies and environments (Grotkopp et al. 2002; Olden et al. 2006). For invasive fish, shifting to a faster life history, reproducing earlier, having more offspring, or offspring that are larger and better able to survive could be beneficial if lifetime is shorter, conditions are novel, and conditions for reproduction are variable from

year to year (Baker et al. 2015). Plasticity in life-history traits could lead to future success of invasives in light of climate change (Olden et al. 2006; Rahel and Olden 2008).

*Heat Shock Proteins*

Another characteristic that could result in invasion success is a fish's ability to tolerate temperature changes. Temperature could be considered the most important abiotic factor contributing to fish invasion success. An organism's ability to maintain physiological function over a wide range of temperatures, what is known as eurythermality, may be stronger in an invasive than in a native species (Zerebecki and Sorte 2011). If an invasive species has a stronger eurythermality than a native species, then it is likely that they will be able to tolerate the stresses of increased temperature due to climate change longer or better than a native species (Zerebecki and Sorte 2011).

One method of of studying physiological temperature tolerance within species is examining heat shock protein expression. Heat shock proteins (HSPs), also known as molecular chaperones, are proteins that help in the refolding of macromolecules that have been denatured due to stress, which ultimately minimizes the cost of protein denaturation and replacement (Zerebecki and Sorte 2011). HSPs are found in all organisms and are characterized based on their molecular weight (kd); e.g. HSP20, HSP70, HSP90 (Kelley 2014). One of the most highly conserved groups of HSP is the HSP70 group and in fish, this group has shown the highest heat shock response (Iwama et al. 1998; Iwama et al. 2004). HSP expression in fish has been measured in various cells and tissues (Iwama et al. 1998). In particular, gill tissue has been shown to be very sensitive to heat stress in fish (Mazur 1996; Ackerman et al. 2000; Basu et al. 2001) and has also been shown to

express HSPs for longer after stress induction (Mazur 1996). The concentration of HSP70 within an organism is directly related to their temperature tolerance (Sorenson et al. 2003; Sorte and Hoffman 2005; Sanders et al. 1991) and HSP expression is thought to be greater in invasive organisms than in natives (Zerebecki and Sorte, 2011). There are 2 types of HSP70 proteins, one that is constitutive (also called HSC70) and one that stress induced (Liang and MacRae 1997). HSC70 is expressed under normal physiological conditions in most cells (D'Souza and Brown 1998) but can also be found at higher levels in organisms that experience high levels of stress often, for example organisms found in tidal zones (Nakano and Iwama 2002; Dong et al. 2008).

## OBJECTIVES

Models of climate change can predict possible outcomes that can result from increasing temperatures, however they cannot properly predict the impacts on populations of invading fish and how non-native species will affect native populations. Climate change is not a process that will happen overnight, so by using comparative studies of life-history strategies of invasive organisms currently living in streams of both warmer and cooler temperatures we can assess the future impacts that climate change may have on these invading fish species. By using the life-history models described by Winemiller and Rose and by using HSP70 response as a measurement of tolerance to thermal stress, I hope to gain insight into the mechanisms of nonnative fish survival in future predicted temperature regimes.

The overarching goal of this project is to understand how different temperature regimes may alter resource allocation of growth and reproduction in invasive brook trout

(*Salvelinus fontinalis*). This project has three objectives. Objective 1) To determine how different stream temperatures impact length and weight at age and timing to maturity in invading brook trout; Objective 2) To determine the impact that temperature has on resource allocation in fish between different streams; Objective 3) To compare stress tolerance, i.e. heat shock response, in adult fish from warmer temperature streams and colder temperature streams.

## MATERIALS AND METHODS

### *Study Organism*

Brook trout are an excellent study organism for studying mechanisms associated with invasion success. They are members of the family salmonidae native to eastern North America (Page and Burr 1991). Brook trout were intentionally introduced to Western North America in the late 1800s and have established populations ranging from Southeast Alaska to Texas (Fig. 1) (Fuller and Neilson, 2014; USGS 2018). They are now the most common trout in small streams in the Western United States (Behnke 1978; Schade and Bonar 2005).

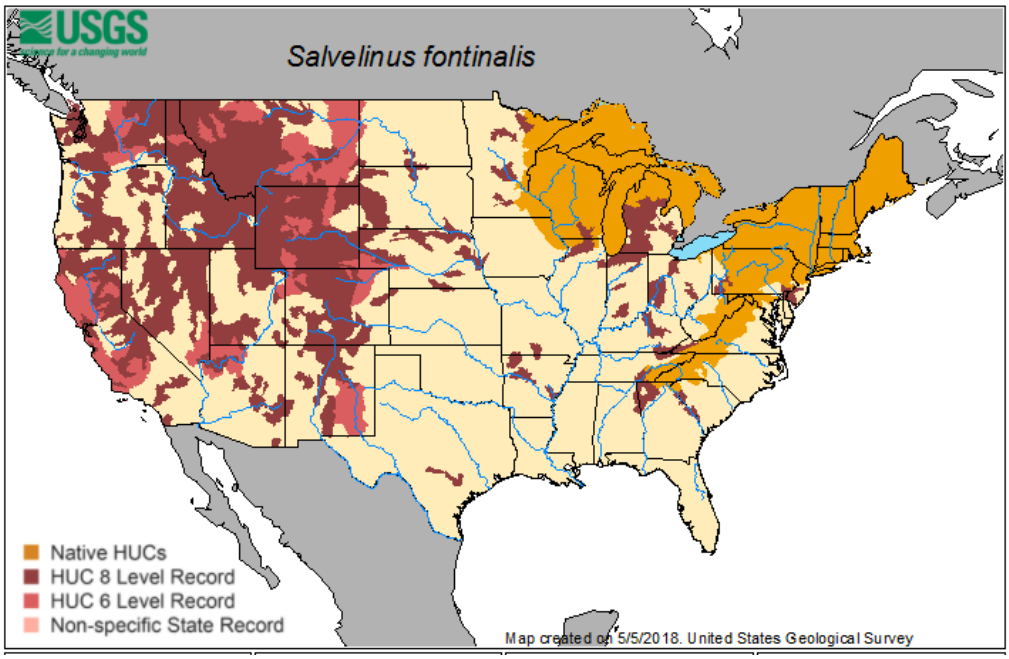



Figure 1. Distribution map of brook trout. (USGS 2018).

Brook trout spawn in the late summer to early autumn (Scott and Crossman 1985), but timing is dependent on a variety of factors including water temperature (Karas 1997) with fish from warmer water temperatures spawning earlier in the season than those from cold water temperatures. They spawn by excavating a nest, or redd, and females deposit between 100 and 5000 eggs depending on their size. Incubation duration is dependent on water temperature, with shorter incubation times in warmer water than colder water (Karas 1997). Larvae hatch mid to late January and will stay within the redd's gravel until their yolk is absorbed, which typically takes 23 to 80 days depending on temperature (Karas 1997). Fully grown adults can reach between 15 to 60 cm and between 0.91 to 6 kg (Karas 1997).

Brook trout can tolerate a wide variety of environmental conditions making them less specialized in terms of habitat demands than other members of the salmonidae family (Karas 1997). These traits have allowed them to invade many different environments. Once established, an invasive brook trout population can easily spread, usually upstream as they have the capacity to travel up steep slopes (>13%) (Dunham et al. 2002), however if sufficient space is limited they will disperse downstream as well (Karas 1997). Given enough time, this allows for a single introduced population to spread through an entire river system (Karas 1997; Dunham et al. 2002). Brook trout invasions displace native cutthroat (*Oncorhyncus clarki*) and bull trout (*Salvelinus confluentus*) populations and are thought to be one of the primary causes of the decline of these populations in western North America (Rieman et al. 2006; U.S. Fish and Wildlife Service 1995; Warnock and Rasmussen 2013), though the mechanisms that cause this are not well understood (Peterson et al. 2004).

Brook trout are a cold water species so are mainly found in cool, clear streams (MacCrimmon and Campbell 1969). The optimal water temperature for growth and survival is between 10.5ºC and 15.5°C (Baldwin 1951; Raleigh 1982; Drake and Taylor 1996). Despite this, brook trout populations in the western part of North America have been found in streams that reach temperatures above 20º C (Baldwin 1951; Raleigh 1982; Drake and Taylor 1996; Kalispel Tribe of Indians, 2016) suggesting a tolerance for a wide range of environmental conditions or an ability to find areas of refuge from increased temperatures.

*Fish Collection*

During October- November 2017, brook trout were collected using backpack electrofishing (Reynolds and Kolz 2012) from eight different streams located within the Pend Oreille river basin: four warmer water streams and four colder water streams. Cold streams include Tacoma Creek (10-16°C), Cee Cee Ah Creek (10-14°C), Le Clerc Creek (10-13°C), and Highline Creek (10-16°C). The warm streams sampled were Cusick Creek (18-20°C), Calispell Creek (18-20°C), and Ruby Creek (18-20°C), and Lime Creek (18-20°C) (Fig. 2). Temperatures used are average maximum temperature for each stream. All fish were then euthanized immediately using 250 mg/L tricaine methanesulfonate (MS-222) and stored at -20°C until further analysis.

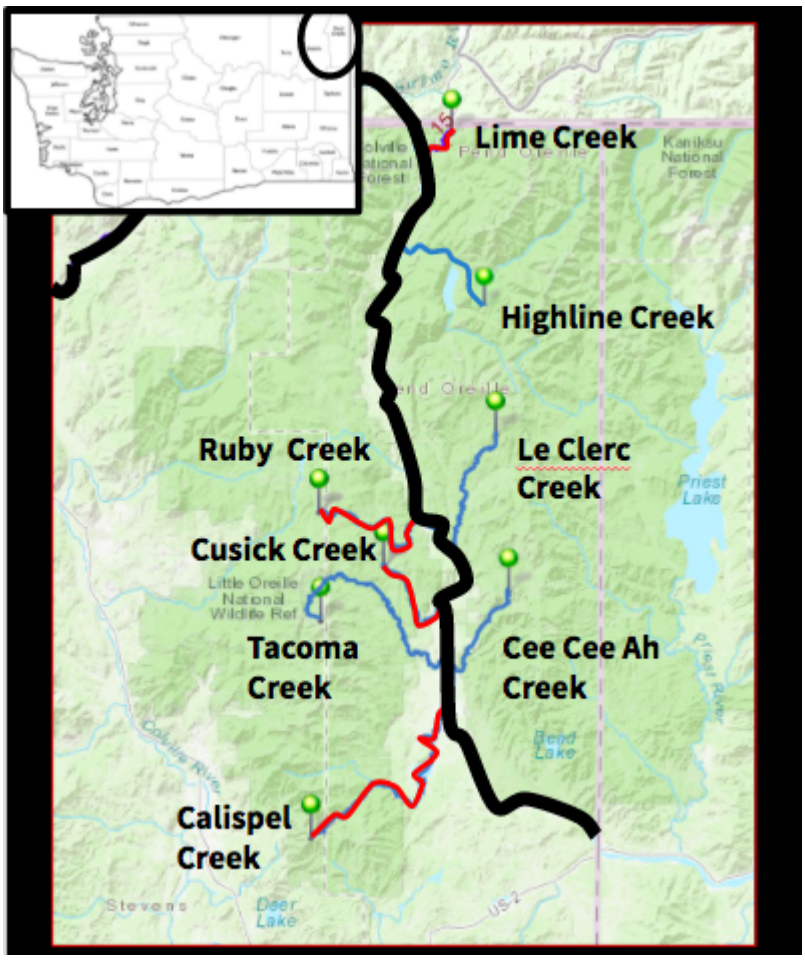



Figure 2. A map of the streams sampled. All streams are located in Pend Oreille County in eastern Washington state. Red lines indicate warm temperature streams and blue lines indicate cold temperature streams. Black line represents the Pend Oreille River.

*Objective 1*

Growth and Timing to Maturity

All fish were measured for total length to 0.1 mm using Vernier calipers and weighed to 0.1 g. Weight and length were used to calculate Fulton's condition factor ($(wt/l^3) \times 100{,}000$) (Neumann et al. 2012). Fish at each stream site were separated by male and female as well as by age in order to account for differences in all categories based on sex and age. Sex of fish was determined through visual analysis and fish that

were not identifiable by visual analysis were classified as unknown. Probability of sexual maturity for fish was measured as a function of total length at maturity for all eight stream locations. Percent of mature fish for each stream at each age was also determined and compared between streams.

Age of fish was determined through analysis of otoliths. Otoliths are calcium carbonate structures in the inner ear that are deposited as annuli in alternating translucent and opaque bands representing periods of fast (summer) or slower (winter) growth (Pine et. al. 2012; Quist et. al. 2012). Otoliths were extracted through base of skull and illuminated under a microscope with isopropyl alcohol and photographs were taken using a Celestron Digital Microscope Imager. Annuli bands were then counted to determine age (Fig 3). Due to difficulty in determining annuli number in fish after age 3, all otoliths with 4 or more annuli bands were determined to be 4+.

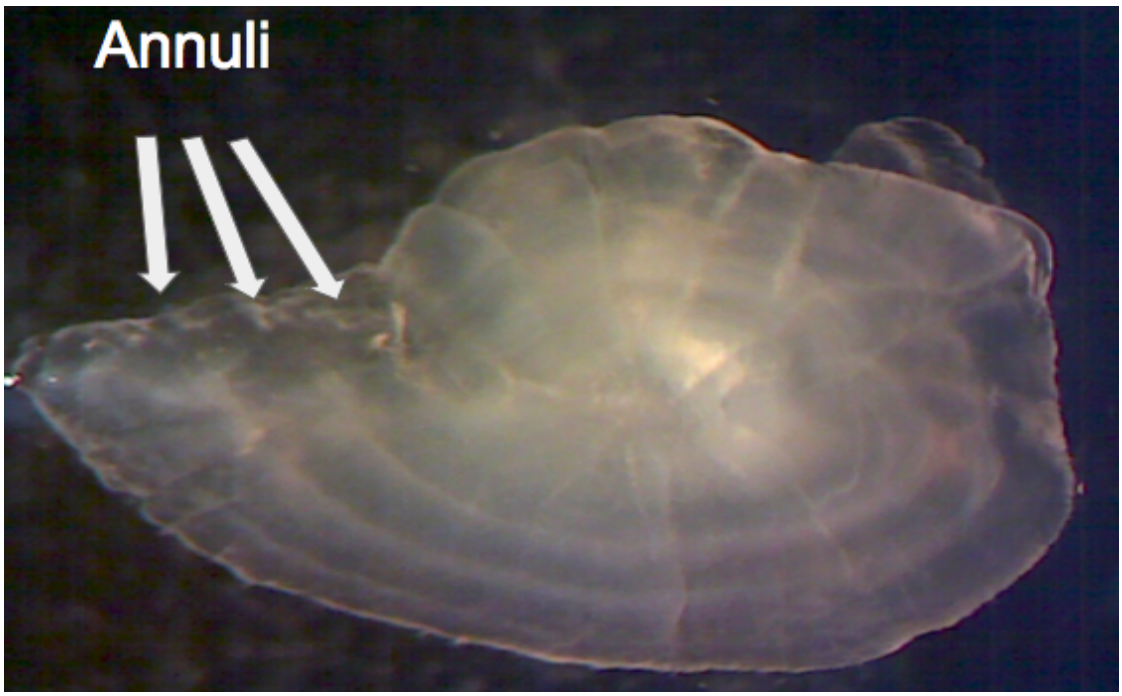



Figure 3. EBT Otolith at 40x magnification. The dark translucent zone represents a period of fast growth (summer) while the white opaque zone represents period of slower growth (winter). Figure shows an age 3 BT.

*Objective 2*

Resource allocation

Gonads from mature males were removed and weighed (g) to determine gonadosomatic index (GSI = [Gonad Weight / Total Tissue Weight] x 100) (Alonso-Fernández et al. 2011). Gonado-Somatic Index is the ratio of gonad weight to body weight and is used to estimate reproductive condition (Flores et al. 2015). Total egg mass from mature females was weighed and individual eggs counted. Diameter and volume of the egg yolk sac ($V=0.1667 \times LH^2$) (Kupren et al. 2014) were measured for 30 eggs from each egg mass.

Lipid Content Analysis

Lipids from all ripe females were extracted and quantified in order to determine female condition after a method from Folch et al. 1957 and Post et al. 2007. First, dorsal muscle tissue was removed from mature females . This tissue was then dried at 50°C in an incubator for 48 hours. Tissue was then ground to a fine powder using mortar and pestle. A 0.5 ∓ 0.0001 g portion of dried tissue was placed into a 30 ml test tube and a 50:50 solution of 8.0 ml chloroform and 8.0 ml methanol were added to bring total volume to 16 ml. This solution was then heated to 60°C until boiled and then cooled to room temperature after which chloroform was added to increase volume to 25 ml. This solution was then filtered through a No. 1 Whatman filter paper into a 125 ml separatory funnel and 10 ml of 0.9% saline was added. The funnel was shaken for 20 seconds and then the solution was allowed to separate and the bottom methanol-chloroform layer was drained into a pre-weighed 50 ml glass beaker. This beaker was then placed on a hot plate at 70°C and the contents were allowed to evaporate for roughly 2 hours. The beaker and its contents were then allowed to cool to room temperature and weighed to the nearest 0.0001 g. This represented total lipid content per 0.5 g of dried tissue.

Statistics

Analysis was done using individual streams as the experimental unit rather than temperature to account for the fact that environmental characteristics between different streams are not uniform. For males, a planned comparisons ANOVA was performed in RStudio to determine between site effects for condition factor at age and GSI at age. For females a planned comparisons ANOVA was performed in RStudio to determine effects

of condition factor at age, lipid content at age, and egg number at age between each stream. Egg weight and egg volume of females were removed from the analysis because variation in the maturation stage of the eggs confounded any differences attributable to environmental factors. Egg number of females was used as the method for determining resource allocation of females. Egg number and lipid content of females was log transformed.

*Objective 3*

Heat Shock Protein Analysis

For objective 3, 8 different ripe male and female brook trout were randomly collected from Calispel Creek (10°C), Cusick Creek (10°C), Ruby Creek (10°C), Tacoma Creek (8°C), Cee Cee Ah Creek (10°C), and Le Clerc Creek (9°C) at the same time as for objectives 1 and 2. Temperatures shown are creek temperatures at the time of sampling. Fish were euthanized using cranial concussion, a Eastern Washington University IACUC approved standard operating procedure for euthanasia. Pairs of gills from each fish were removed. Gills were flash frozen using dry ice and ethanol slurry and were stored at -80℃ until further analysis. Gill tissue was weighed to 100 mg and rinsed with 1X phosphate buffered saline (PBS) followed by two freeze-thaw cycles to homogenize the cell membranes following manufacturer's protocol.

Proteins were analyzed using an enzyme-linked immunosorbent assay (ELISA) (My BioSource MBS706016) which uses anti-fish Hsp70 antibodies that were generated in mice and the antibodies measure both constitutive and inducible forms of Hsp70 (Padmini et al. 2015; Ekabaram and Narayanan 2016). Absorbance was read at 450 nm

wavelength on a SPECTRAmax 250 plate reader. Samples were run in triplicate and distributed randomly over two 96-well plates provided with the kit. A Bradford assay (Bradford 1976) was used to determine nanograms of total soluble protein (ng TSP). HSP70 was expressed as pg HSP70 per ng of TSP.

Hsp70 concentration was calculated with reference to a standard curve of Hsp70 protein (from 18.75 pg/ml to 300 pg/ml) that was run on each plate. HSP70 levels are expressed as pg HSP70/ng TSP. Statistical difference of average pg of HSP70 between streams was determined by running a Kruskal-Wallis rank sum Test in order to take into account differences in both temperature and stream as a factor of heat shock response rather than just temperature.

## RESULTS

### *Growth and Timing to Maturity*

Comparisons between fish from warm vs cold streams showed no significant difference for fish condition factor between streams with the exception of fish from warm creek Cusick, which were significantly smaller than fish from cold creek Highline at age 0 and 2, fish from Calispel, which were significantly smaller than fish from Highline at age 1, and fish from warm creek Lime. Condition factor for fish from Lime was significantly smaller than condition factor for fish from all other streams, including all other warm streams, at ages 0-3 besides fish from cold creek Tacoma at age 2 (Fig. 4 and Table. 1) and was only significantly smaller than fish from warm creek Ruby at age 4+.

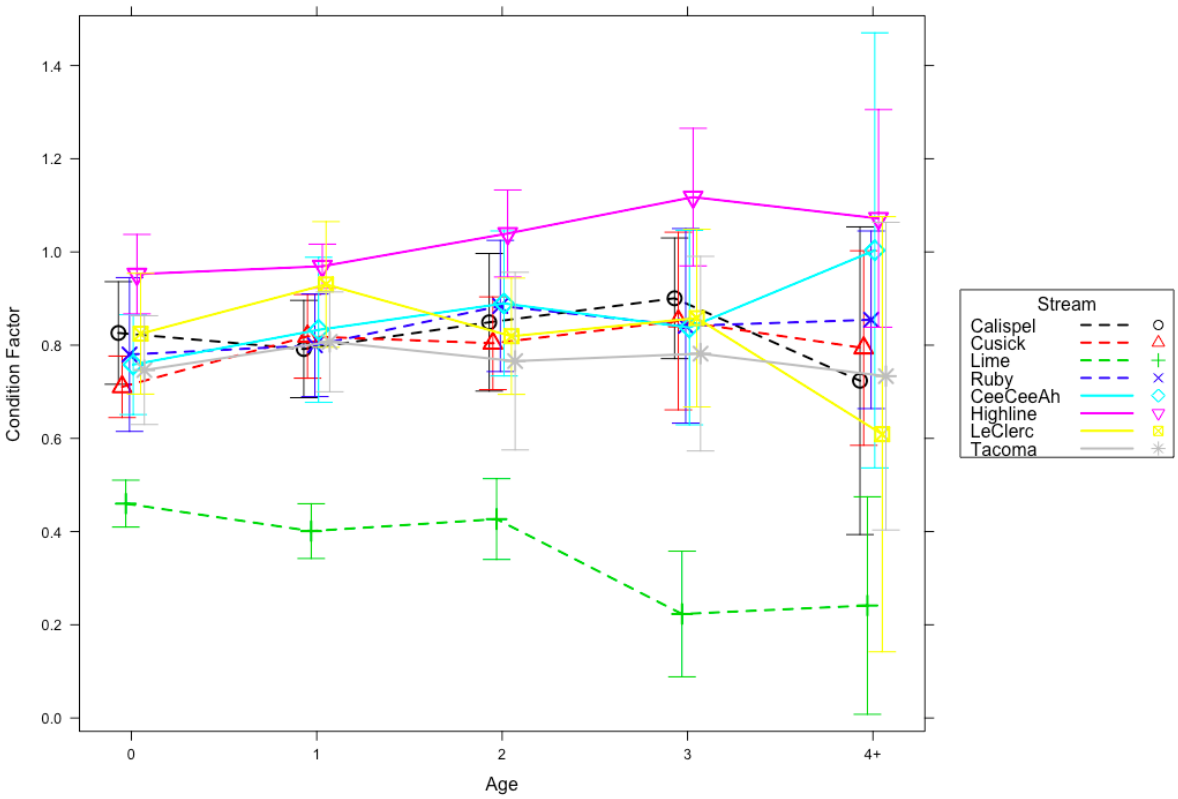
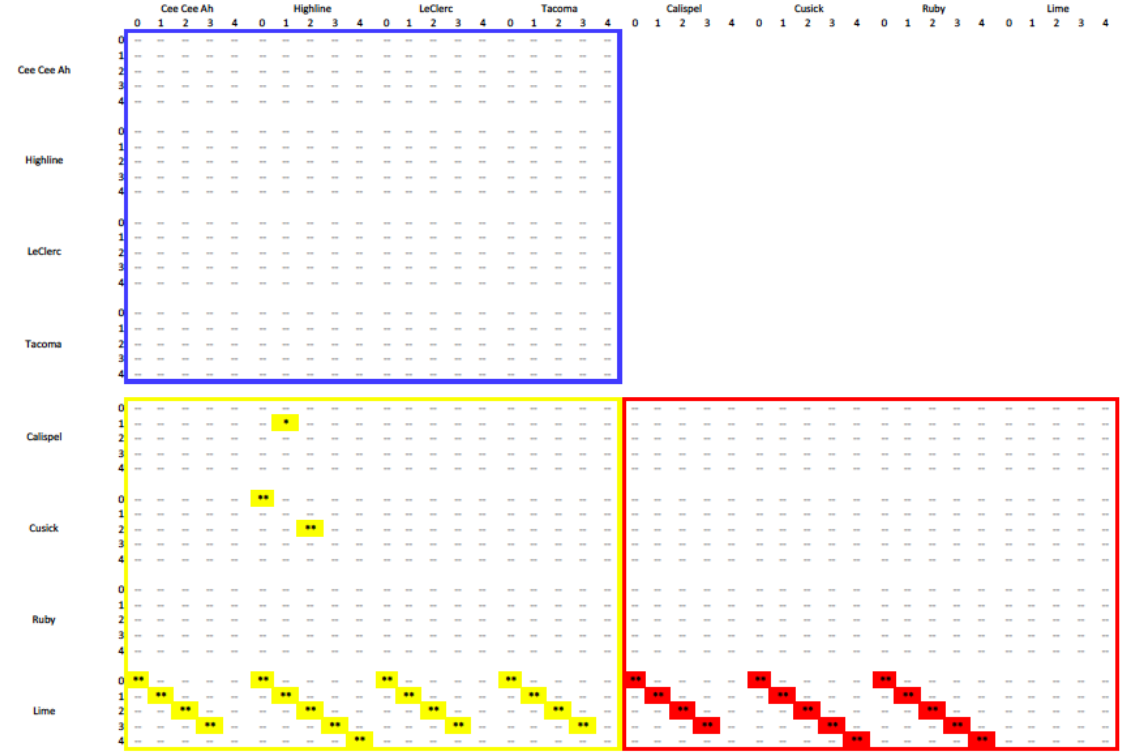



Figure 4. Fish condition factor at age between all eight stream locations. The 4+ category includes all fish that were determined to be ≥4. Dashed lines represent warm streams, solid lines represent cold streams.

Table 1. Significance value for fish condition factor at age for all eight stream locations using an ANOVA with Tukey's adjustment. * denotes P-Value < 0.05, ** denotes P-Value < 0.01. -- denotes no significant value. Blue box represents significant difference between cold streams, red box represents significant difference between warm streams, and yellow box represents significant difference between cold and warm streams.




Significant differences for probability of sexual maturity for fish as a function of length were seen (Fig. 5). Fish from warm creek Lime were significantly smaller at maturity than fish from Calispel, Ruby, Highline, Tacoma, and Le Clerc Creeks. Fish from warm creek Cusick were also significantly smaller at maturity than fish from cold creek Tacoma. A trend was seen where fish maturity level declines toward larger length for Lime Creek, Cusick Creek, and Highline Creek.

Fish age at maturity varied between sex and streams (Fig. 6 and Fig. 7). Male maturity at age 0 was observed for warm creeks Cusick and Lime and cold creek

LeClerc. Male maturity at age 1 was seen for all streams except Tacoma Creek. Female maturity at age 1 was seen for Cusick, Calispel, Lime, Highline, and LeClerc Creeks. Both males and females again showed a decrease in percent mature at age 4+, with some females showing a decrease at age 3.

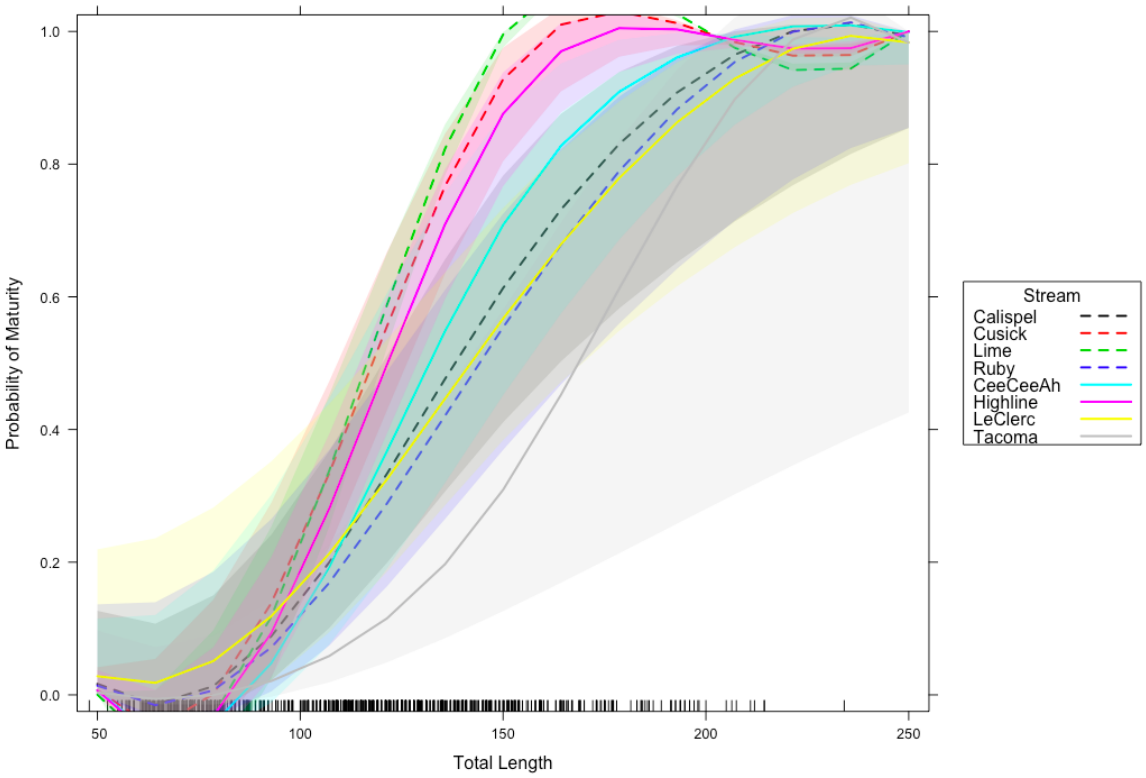



Figure 5. Probability of sexual maturity as a function of total length for fish from all eight stream locations. 0 represents not mature and 1 represents mature. Dashed lines represent warm streams, solid lines represent cold streams.

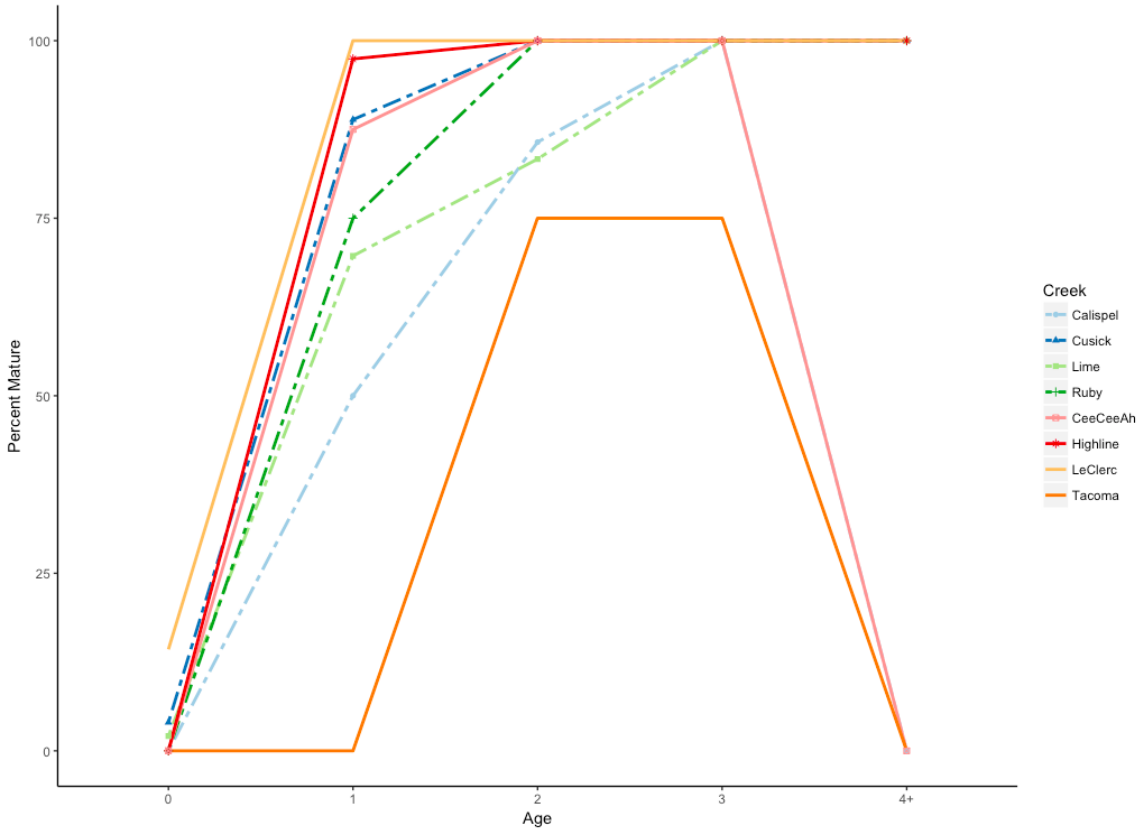



Figure 6. Percent of mature males at each age for all eight stream locations. Dashed lines represent warm streams, solid lines represent cold streams.

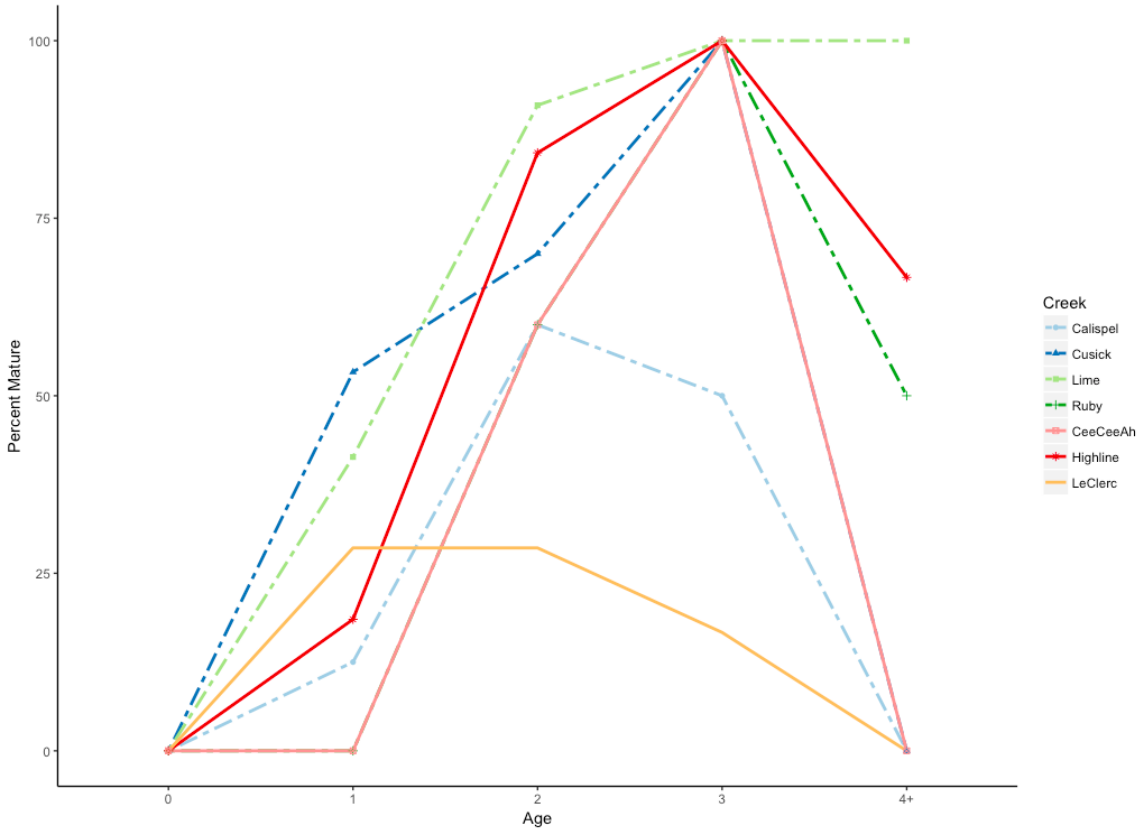



Figure 7. Percent mature females at each age for different stream locations. Solid lines represent cold creeks and dashed lines represent warm creeks. No ripe females were found at Tacoma Creek so it was removed from the analysis. Dashed lines represent warm streams, solid lines represent cold streams.

*Resource Allocation*

Fish from Highline Creek showed a significantly higher GSI than fish from Cusick at ages 1 and 2 (Figure 8). Fish from Highline Creek also had a significantly higher GSI than fish from LeClerc Creeks at age 2 and fish from Tacoma Creeks at age 3. No males with ripe gonads were found for Lime at age 4+.

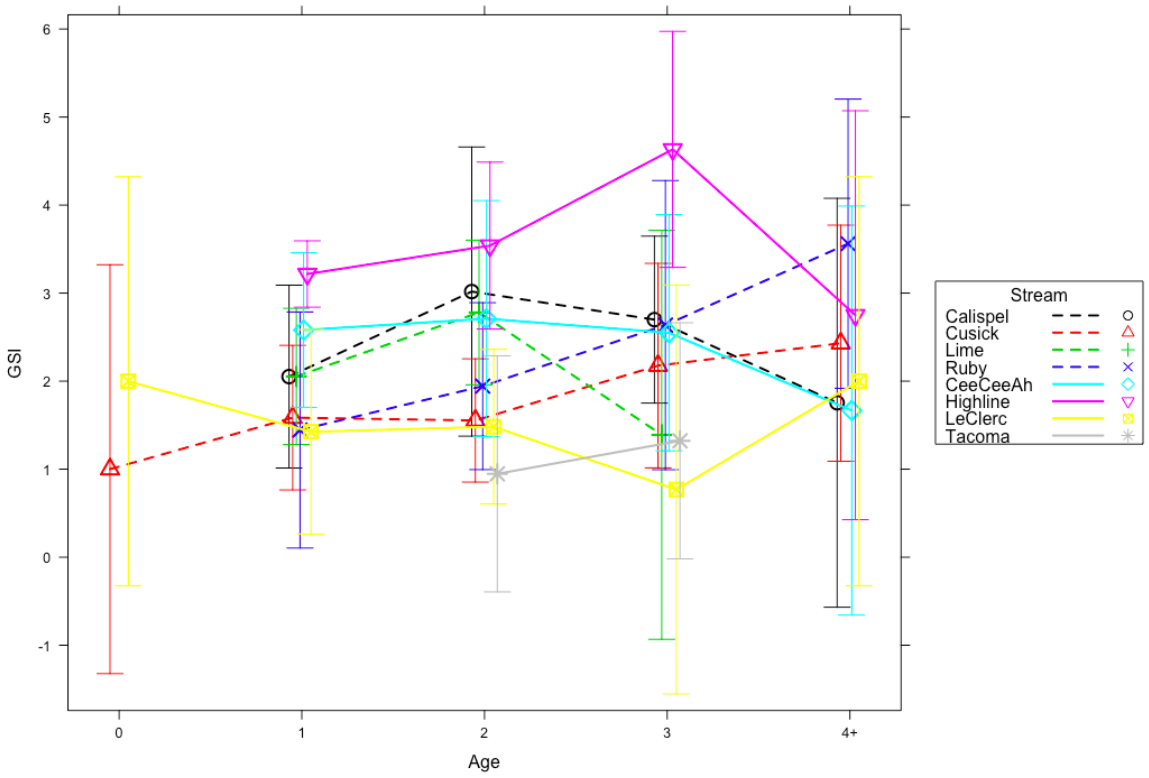



Figure 8. GSI at each age from different stream locations. All fish represented are males. Age 4+ represents all fish that were found to be ≥ age 4. Dashed lines represent warm streams, solid lines represent cold streams.

For females, lipid content was not found to be significantly different between streams (Fig. 9). No ripe females were found from Tacoma Creek, so it was removed from lipid content and egg number analysis.

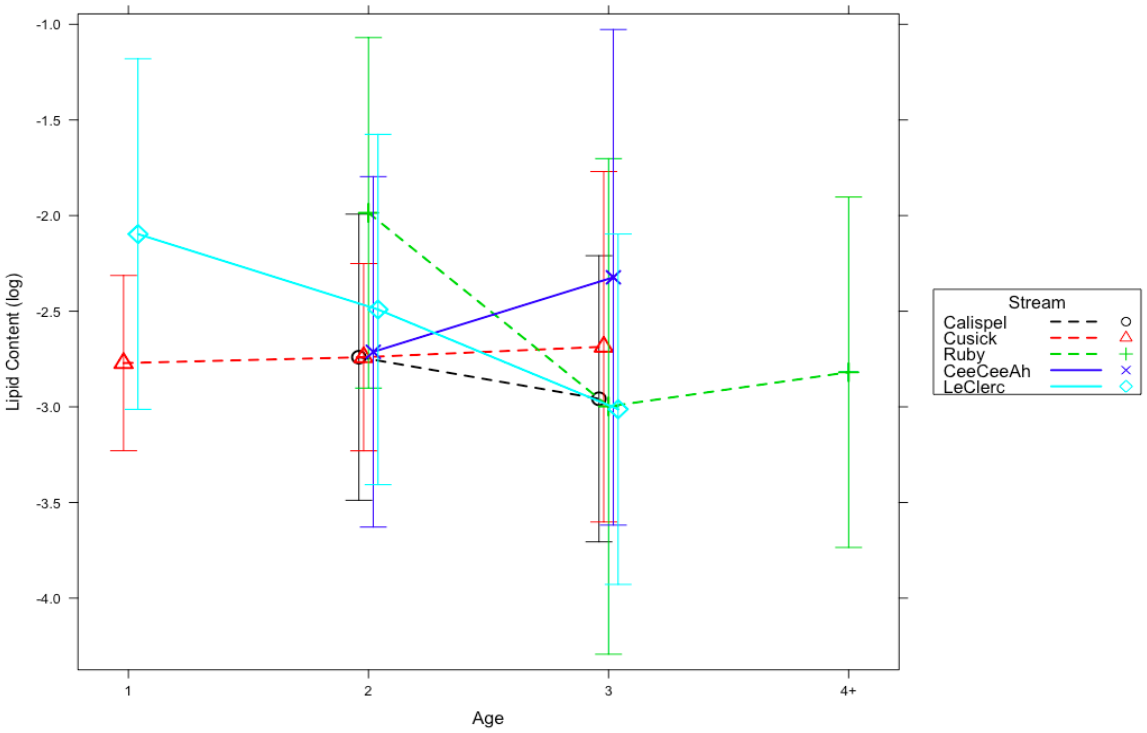



Figure 9. Lipid content at each age for different stream locations. All fish represented are females. Numbers have been log transformed. Age 4+ represents all fish that were found to be ≥ age 4. Dashed lines represent warm streams, solid lines represent cold streams. No ripe females were found for Tacoma Creek so it was removed from the analysis. Lipid content was not analyzed for Lime and Highline Creek.

Egg number at age of females varied in significance depending on stream site as well as age of females (Fig. 10 and Table 2).

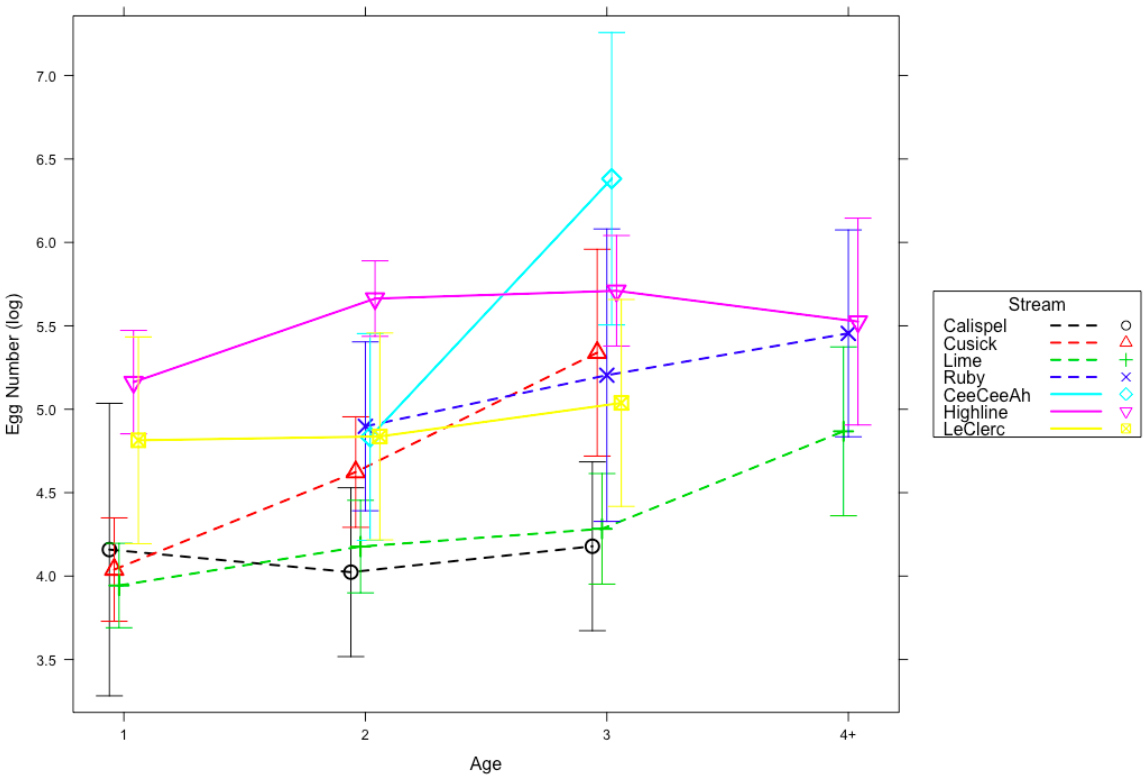
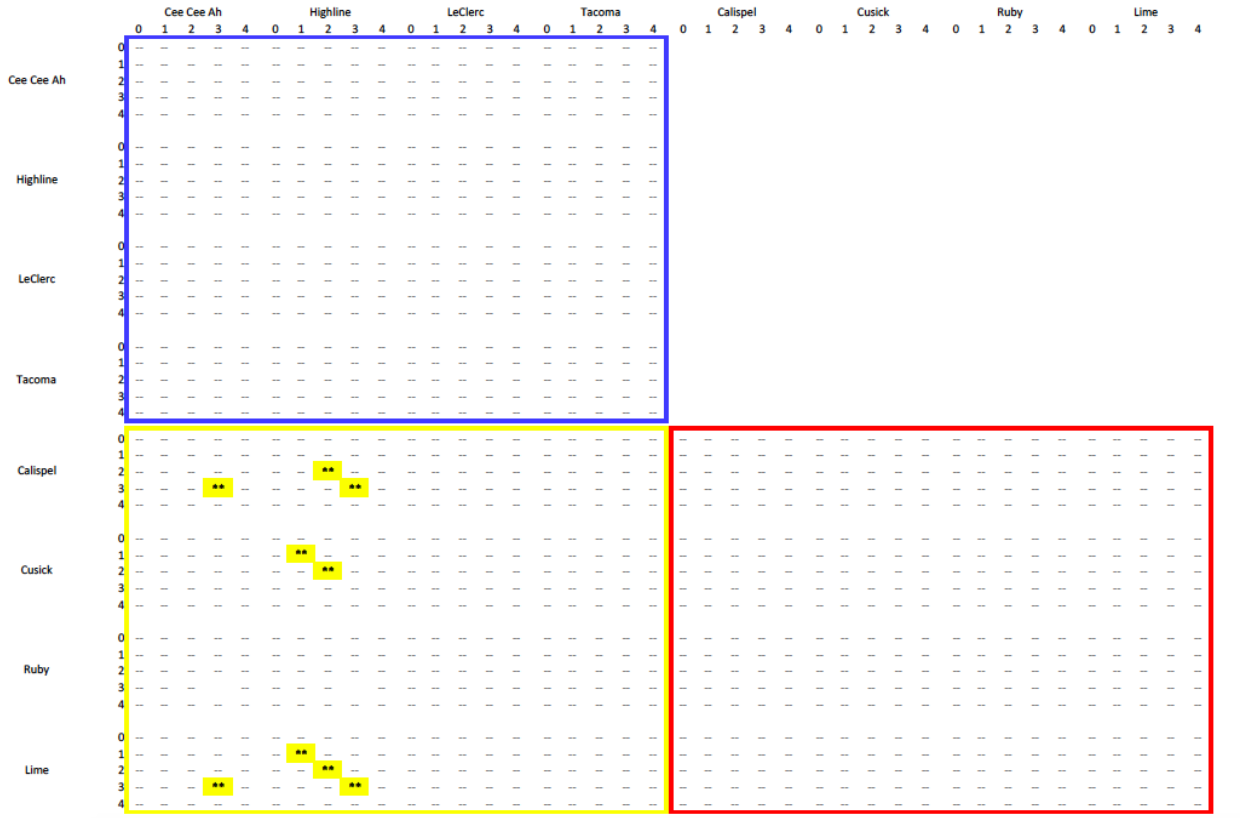



Figure 10. Egg number for females at each age for different stream locations. Egg numbers have been log transformed. Age 4+ represents all fish that were found to be ≥ age 4. Dashed lines represent warm streams, solid lines represent cold streams. No ripe females were found at Tacoma Creek so it was removed from the analysis.

Table. 2. Significance value for fish egg number at age for all eight stream locations using an ANOVA with Tukey's adjustment. * denotes P-Value < 0.05, ** denotes P-Value < 0.01. -- denotes no significant value. Blue box represents significant difference between cold streams, red box represents significant difference between warm streams, and yellow box represents significant difference between cold and warm streams.




Females from colder creeks Highline and Cee Cee Ah both showed significantly higher egg number than warmer streams Calispel and Lime, but this was dependent on age. Egg number from females at Lime Creek were significantly less than Highline Creek at age 1, 2, and 3 and significantly less than Cee Cee Ah at age 3. Egg number from females at Calispel Creek were significantly less than Highline Creek at age 2 and 3 and was significantly less than Cee Cee Ah at age 3. Egg number from females at Cusick Creek were significantly less than Highline Creek at ages 1 and 2. No females with eggs

were found for Cee Cee Ah at age 1 or 4, Ruby at age 1, and LeClerc, Cusick or Calispel at age 4.

*Heat Shock Proteins*

There was no significant difference between amount of gill HSP70 in fish in warm and cold streams. There was however, more variation seen between fish gill HSP70 in colder streams, with fish from LeClerc Creek showing significantly lower average gill HSP70 per ng of TSP than all other streams except warm creek Calispel (Fig. 11).

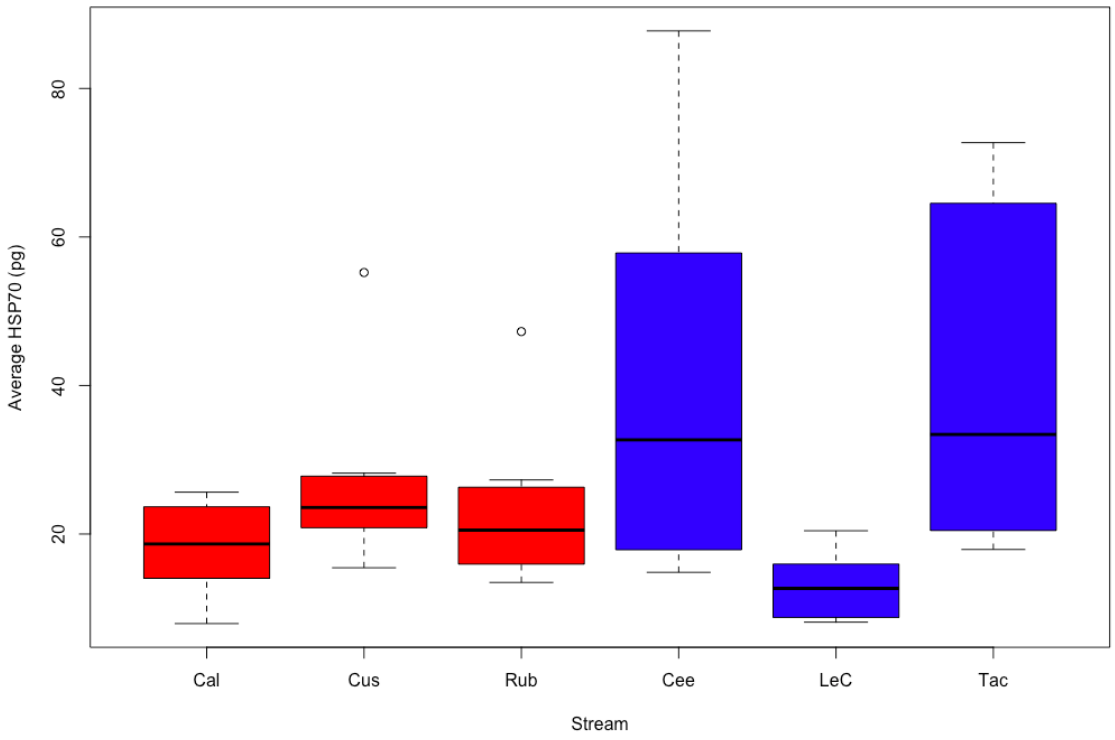



Figure 11. Average pg of HSP70 per ng of TSP for fish from all six streams analyzed. Analysis of HSPs was not done for Lime or Highline Creeks. The 3 streams on the left are warm water streams, the 3 streams on the right are cold water streams.

## DISCUSSION

The goal of this project was to determine the impact that higher water temperatures have on the life history traits and resource allocation of invasive eastern brook trout. Though temperature was shown to have some impact, especially on female egg number, most differences seen did not seem to be a result of temperature change, and could be impacted by other environmental factors or complex interactions among multiple factors.

### *Growth and Timing to Maturity*

In this study, fish condition factor at age varied in significance depending on stream site as well as age, but no consistent patterns were seen when comparing cold water streams to warm water streams. Fish from Lime Creek had a significantly lower condition factor than fish from all other streams, but this was dependent on age with some ages showing significance with some streams while other did not. Lime Creek has a substrate made of degraded limestone. It is a very shallow stream that is slow flowing, low gradient, with moderate beaver abundance. Perhaps in this stream nutrient composition is limited leading to increased competition and therefore growth is disrupted, though these habitat factors were not analysed in this study.

Based on the data collected, no consistent relationship was observed between temperature and fish growth rate. Temperature has been shown to impact feeding rates of salmonids, especially as temperatures increase above the preferred temperature range (Linton et al. 1998). As temperatures increase feeding rate increases, but as temperatures rise above the preferred temperature range, growth potential decreases (Jobling 1981,

Linton et al. 1998). Therefore you would expect that in the warmer temperature streams sampled in this study, stress would cause a decrease in growth. The results from this study contradict this hypothesis and this may be because other factors besides temperature are contributing to brook trout growth within these streams.

Significant differences for probability of sexual maturity for fish as a function of length were seen, however this did not seem to be a result of temperature. Fish from warm creek Lime were significantly smaller at maturity than fish from both warm and cold streams (Warm: Calispel and Ruby; Cold: Highline, Tacoma, and Le Clerc Creeks). Fish from warm creek Cusick were also significantly smaller at maturity than fish from cold creek Tacoma however this pattern was not consistent among other warm and cold streams so perhaps other environmental factors are impacting the lengths at maturity in this stream. The dip in maturity shown in figure 5 for fish from Highline, Cusick, and Lime at larger body sizes suggest that in these streams, fish may be alternating spawning years but this would need to be analyzed further. An interesting pattern was seen for time to maturity between streams with males maturing at age 0 in 3 of the 8 streams and maturity at age 1 for all streams except Tacoma Creek. Female maturity also showed an interesting pattern with females from 3 of 4 warm creeks and 2 of 4 cold creeks showing maturity at age 1. Brook trout in their native range typically spawn at 2+ years (U.S. Fish and Wildlife Service 2018) which could suggest a shift in life history strategy toward early maturation for these invasive populations.

Tacoma Creek showed no ripe females at any age due to the fact that most females that were old enough to spawn had either already spawned or were not ready to spawn this season. This then shows possible variation in timing of spawning between

different streams, though this was not analyzed. Tacoma Creek is a cold Creek that is faster flowing with little to no competition from other species (visual observation, 2017) so perhaps temperatures for spawning are reached earlier in this stream than others, or since there are higher nutrients, females can wait to spawn between different years if offspring survival is high.

*Resource Allocation*

Significant differences were found for male fish GSI between different streams but this was seen between cold and warm streams as well as from cold and cold streams suggesting there is no temperature effect on male GSI. No significant differences were seen for lipid content at age for females between warm and cold streams which is not surprising since condition factor for females was also not significant between streams. Egg number at age for females, however, varied in significance depending on stream site as well as age. Overall females from colder streams Highline and Cee Cee Ah produced more eggs per spawning season than females from warmer streams Calispel, Cusick, and Lime, but this was dependent on age. Given that condition factors for fish were not different between these streams, this is a surprising result as egg number is typically closely associated with female length (Baker et al. 2008; Goncalves et al. 2011). Therefore there may be a trend that females in colder streams, regardless of size are producing more eggs, whereas females from warmer streams, regardless of size are producing less eggs.

Egg number represents the maximum number of offspring produced from a single reproductive event and is therefore considered one of the most fundamental life-history traits (Baker et al. 2015). Egg number typically shows a tradeoff with egg size (Baker et al. 2015), but due to difficulty in determining yolking stage of unfertilized eggs, egg size was removed from this analysis. Hypothetically, if egg size is increased due to the environment, then there must be a plastic decrease in egg number in order to account for the increased cost of reproduction, which could account for the low number of eggs seen in sampled streams. Warmer streams showed a lower number of eggs per female, suggesting that fish in warmer streams are producing larger eggs which hypothetically could result in higher survivability of offspring, though this was not analyzed.

*Life History Shifts*

Common length for adult brook trout in smaller streams is between 150-330 mm (Karas 1997; U.S. Fish and Wildlife Service 2018), however adult fish found in study streams here were between 100-260 mm. This suggests a decrease in length for invasive populations in all streams regardless of temperature. Also, brook trout normally deposit between 100-5000 eggs depending on their size (Karas 1997). In the streams sampled for this study, however, fish showed a much smaller number of eggs ranging from 20-300 eggs. According to the life-history model of Winemiller and Rose data from this study suggest that these invasive populations are exhibiting an opportunistic strategy where they are typically smaller, mature earlier and have low fecundity per spawning event.

*Heat Shock Proteins*

HSP70 has been shown to be a mechanism for higher thermal tolerance in fish and may be increasing the temperature tolerance of invasives (Zerebecki and Sorte 2011). In this study, no significant differences were found between heat shock expression in warm and cold streams. These results could suggest that brook trout in warmer streams are finding areas of refuge during high summer temperatures and so do not express higher levels of HSP70. There has also been some suggestions the amount of time higher HSP is expressed as well as the degree of increase in HSPs can be influenced by past thermal acclimation (Deane and Woo, 2011 ; Chadwick et al. 2015). This could then explain the variation seen in amount of HSP70 for fish from cold water streams with fish from LeClerc Creek showing a lower average of HSP70 per pg of total soluble protein than fish from both Tacoma and Cee Cee Ah Creek. The variation in HSP70 expression shown from fish in colder streams could be due to higher stress during capture. This could suggest that brook trout in warmer streams are more tolerant of stress than those found in colder streams, though this would need to be analyzed further.

*Plasticity of invasives*

Establishment and spread of invasive species within a novel environment is dependent upon the species ability to maintain a high level of performance over a wide range of environmental conditions (Cortes et al. 2016). In order for a species to successfully invade they must first pass through the new environments "ecological filter" composed of both an abiotic as well as a biotic component. A novel species in a new habitat must be able to maintain performance and fitness in terms of species interactions

including competition and predation but must also tolerate abiotic stressors such as temperature through physiological adjustments (Rahel and Olden 2008; Cortes et al. 2016). Fish analysed in all streams here show so much variation within each stream that it is negating any significant effect. Perhaps invasive fish already exhibit so much plasticity in order to successfully invade a new habitat that they are able to quickly respond to changes within an environment.

*Conclusion*

The results of this study suggest that invasive populations of eastern brook trout may be capable of quickly switching to faster life histories as a result of novel habitat, regardless of temperature stress. This could either be through plasticity or through adaptation, but more work would need to be done in order to determine this. One interesting study would be to determine the plasticity in life-history of native brook trout compared to invasive brook trout to determine if the invasive form of brook trout shows more plasticity or adaptation in life-history. Our data demonstrate that brook trout in their invasive range could sustain healthy populations in the face of climate change due to life-history plasticity, though the insignificant results between warmer and colder streams from the HSP analysis could suggest that brook trout are capable of finding refuge from higher stream temperatures in summer months. There were no consistent significant differences found for fish between warm and cold streams for growth, GSI, lipid content, and timing to maturity which suggests some other factors may be contributing to these measures within these streams. This could include factors such as brook trout habitat length, wetted width of streams, beaver activity in streams, flow rate and gradient scale,

and presence of other piscivorous trout. The effects of these factors were not analysed for this study but will be the subject of future investigation. Differences in egg number for females between warmer and colder streams suggest some impacts of temperature on brook trout fecundity, though survivability of eggs is unknown. Perhaps in warmer temperatures, females are producing larger eggs in order to account for stresses due to higher temperatures and resulting in a smaller size of all eggs. This could increase survivability of offspring in stressful temperatures. Future climate change models are leading to concern for further range expansion of brook trout into bull trout range and possible extirpation of bull trout (Rieman et al. 2007; Warnock and Rasmussen 2013) and the results of this study support those models. Knowledge of life-histories between brook trout in different streams is important to have in order to inform suppression efforts of eastern brook trout. Since brook trout in different streams have varied strategies, understanding the dynamics of each population is important for accurate modeling of different populations and suppression efforts.

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